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published in

Ecosystems

2019

DOI (link to publisher)

[10.1007/s10021-019-00351-3](https://doi.org/10.1007/s10021-019-00351-3)

document version

Publisher's PDF, also known as Version of record

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citation for published version (APA)

Chang, C., Wu, F., Wang, Z., Tan, B., Cao, R., Yang, W., & Cornelissen, J. H. C. (2019). Effects of Epixylic Vegetation Removal on the Dynamics of the Microbial Community Composition in Decaying Logs in an Alpine Forest. *Ecosystems*, 22(7), 1478–1496. <https://doi.org/10.1007/s10021-019-00351-3>

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Effects of Epixylic Vegetation Removal on the Dynamics of the Microbial Community Composition in Decaying Logs in an Alpine Forest

Chenhui Chang,^{1,3} Fuzhong Wu,^{1,2} Zhuang Wang,¹ Bo Tan,^{1,2} Rui Cao,¹ Wanqin Yang,^{1,4*} and Johannes H. C. Cornelissen³

¹Long-term Research Station of Alpine Forest Ecosystems, Institute of Ecology & Forestry, Sichuan Agricultural University, Huimin Road 211, Wenjiang District, Chengdu 611130, People's Republic of China; ²Collaborative Innovation Center of Ecological Security in Upper Reaches of Yangtze River, Chengdu 611130, People's Republic of China; ³Systems Ecology, Department of Ecological Science, Faculty of Science, Vrije Universiteit, Amsterdam, Amsterdam, The Netherlands; ⁴School of Life Science, Taizhou University, Shifu Road 1139, Taizhou 318000, People's Republic of China

ABSTRACT

Epixylic vegetation may be important in dead wood decay by altering the microenvironment and, thereby, microbial communities in logs. However, the interaction between epixylic vegetation and dead wood microbial communities remains poorly known. Therefore, repeated experimental epixylic (bryophyte-dominated) vegetation removal (ERM) from logs of the fir *Abies faxoniana* across a wide range of decay classes (I–V) was conducted on the eastern Tibetan Plateau. The dynamics of the microbial community were separately measured in heartwood, sapwood and bark using the phospholipid fatty acid analysis (PLFA) method. Our results showed that the effects of ERM on the microbial community depended greatly on the three log components and sampling seasons but less on decay class. (1) The absence of epixylic vegetation

generally enhanced the total microbial biomass and Sørensen similarity in bark, whereas it had a more complicated effect on those in heartwood and sapwood. Specifically, the response to ERM became progressively stronger from winter until the late growing season. (2) ERM increased the total percentage of Gram-negative bacteria and fungi in heartwood and upper side sapwood and decreased their percentages in bark. (3) The moisture content and pH of the logs were good predictors and likely drivers of the dynamic patterns of the microbial community composition. Our findings demonstrate strong and partly consistent interactions between epixylic vegetation and microbial communities. Further in-depth research should reveal how these interactions feed back to the decomposition process of logs and thereby to carbon and nutrient cycles in the alpine forest ecosystem.

Key words: *Abies faxoniana* Rehder & E. H. Wilson; bacteria; bark; dead wood; decay class; epixylic vegetation removal; fungi; seasonal snow cover.

Received 26 August 2018; accepted 21 January 2019;
published online 5 March 2019

Electronic supplementary material: The online version of this article (<https://doi.org/10.1007/s10021-019-00351-3>) contains supplementary material, which is available to authorized users.

*Corresponding author; e-mail: scyangwq@163.com

INTRODUCTION

Coarse woody debris (CWD) and associated epixylic vegetation are crucial to carbon and nutrient cycles and energy flow (Harmon and others 1986; Deane-Coe and Stanton 2017) in forest ecosystems. CWD decay and epixylic vegetation influence each other. Most prior studies have concentrated on the effect of CWD decay class (DC) on epixylic abundance, diversity and community composition (Ódor and others 2006; Spribille and others 2008; Kumar and others 2018). However, theoretically, epixylic bryophytes and lichens can, in their turn, influence the decay process of logs by altering the decomposition environment and the microbial communities in the log (Shorohova and others 2016; Romashkin and others 2018). It is obvious that, in the case of 'buried wood,' for example, in peaty forests, vegetation growing on or around logs can have a huge effect on their fate, primarily by inhibiting their decomposition (Hagemann and others 2010; Moroni and others 2015). However, in many forests, the logs remain largely above the soil surface when downed naturally or after forestry operations. Moreover, the colonization of a log on the surface by epixylic vegetation is a long and dynamic process, with the succession of species paralleling the process of log decay (Rajandu and others 2009). It is well known that the succession of dominant species usually starts with epiphytes and ends with epigeous species due to the changes in log structural and chemical properties (Kushnevskaia and others 2007) and the time available for colonization and succession. Consequently, the ecological function of the epixylic community and the associated decomposer community (Bartels and Chen 2015; Kumar and others 2018) should vary with epixylic vegetation succession stage (decay classes). Although these relationships have been commonly assumed, they are poorly documented, and the feedback loop between tree log decay class, their epixylic vegetation and the microbial communities in the logs remains poorly understood.

Wood decomposition is considered to be carried out gradually by multiple microbial taxa, that is, fungi, Gram⁺ bacteria, Gram⁻ bacteria and non-fungal micro-eukaryotes (Rayner and Boddy 1988; Brown and Chang 2014; Rajala and others 2015), and these taxa differ in their preference for and efficiency of degradation of different compounds of wood (Bugg and others 2011; Bani and others 2018). Specifically, fungi are considered the most important participant in degrading recalcitrant ingredients and can be affected by the substrate

moisture and pH, while bacteria can break down both more and less recalcitrant forms of organic matter depending on the taxon and environmental regime and can affect fungal decomposition (Cornelissen and others 2012). Thus, changes in microbial composition and activity in terms of those higher taxa may significantly affect the wood decay rate (Hoppe and others 2015, 2016). The principal drivers (temperature, moisture content and substrate quality) of microbial decomposition (Harmon and others 1986) could be influenced by the presence of bryophytes and lichens on decomposing logs, for instance by covering foliar litter and influencing its decomposition (Sedia and Ehrenfeld 2006; Asplund and Wardle 2017). However, aside from anecdotal observations (the possible effects of epixylic species on log decay via microbial community) (Harmon and others 1986; Dynesius and others 2010), we know very little about such relationships for logs [but see Hagemann and others (2010)], partly because the direction of causality of log microclimate versus bryophyte cover and composition cannot be verified without experimental manipulation (Harmon and others 1986; Dynesius and others 2010; Ando and others 2017). However, how and the extent to which epixylic vegetation influences microbial communities and their biomass in decaying logs, via these or other mechanisms, is still not well understood.

The effects of epixylic vegetation on wood decay may vary with the structural component of the log (that is, heartwood versus sapwood versus bark), seasonal environment and substrate decay class, all of which may control the biomass and composition of epixylic species. Compared with the relatively smooth surfaces of exposed heartwood and sapwood (whether at cut surfaces or after bark has fallen off), the rougher bark surface can provide cryptogams with a more suitable habitat for colonization (Ódor and others 2013). Furthermore, the lower part of logs is in contact with the soil and may be more susceptible to the colonization of mosses and other cryptogams; thus, epixylic vegetation may first colonize the bark and the lower exposed parts of logs. The distribution, abundance and function of epixylic vegetation can also vary with seasonal changes in temperature, precipitation and air humidity. Crown epiphytes have been found to have different roles in seasonal nutrient dynamics by gathering nutrients in dry winters and promoting the leaching of nutrients in rainy summers (Parker 1995); parallels may be expected in epixylic vegetation. Seasonal variations in microbial communities have also been found in epiphytic foliage, which were associated with herbivore-ac-

companied injection and epiphytic tissue senescence (Davey and others 2012). Again, the effects of epixylic vegetation on the microbial community may vary seasonally according to the same mechanism. Moreover, the distribution, cover and thickness of epixylic vegetation may also vary with the decay class (Rajandu and others 2009; Stokland and others 2016). The more advanced the decay class, the more time available for epixylic species to colonize the substrate and expand. Epixylic species are also closely related to the looseness and texture (fissure index) of the bark and the water holding capacity, density and pH of the wood or bark substrate (McAlister 1997; Fukasawa and others 2015). All these traits differ substantially not only among tree species (Zuo and others 2016) but also among decay classes (Chang and others 2015); for instance, wood in a more advanced stage of decay tends to be less dense and moister, providing a favorable substrate for epixylic species. However, knowledge about how epixylic vegetation in turn affects decaying logs via its effects on the composition of microbial organisms under the influence of these environmental factors and substrate traits is lacking. Unraveling these relationships is the objective of our systematic study.

Therefore, we hypothesized that (1) the microbial community composition in heartwood and sapwood would respond distinctively to the presence versus absence of epixylic vegetation compared to the response in bark; (2) the effects of epixylic vegetation presence versus absence on the microbial community composition in terms of the higher taxa would be affected by the combination of log decay class and environmental conditions in different seasons of the year; and (3) the responses of microbial community composition to the presence versus absence of epixylic vegetation would be closely related to the altered microclimate and pH of the log.

We tested these hypotheses in alpine fir forests distributed across the eastern Qinghai–Tibet Plateau and the upper reaches of the Yangtze River. This region plays important roles in holding fresh water, sequestering carbon and indicating climate change (Wang and others 2015; Xiao and others 2016) (Figure A1). Bryophytes and lichens in this high-altitude region are also facing considerable global environmental changes, such as increased temperature and nutrient availability, which may lead to perturbation in the composition of cryptogams. The presence versus absence of epixylic bryophytes and lichens, especially on decaying logs, may influence wood decay dynamics and may thus affect carbon stocks in cold-biome forest sys-

tems. Studying the effects of epixylic vegetation on wood decomposition is a long-term process, especially in alpine forests with their harsh climate. The key innovation of our study is to systematically disentangle the sequential responses of the microbial community to the presence versus absence of epixylic vegetation cover along the whole log decay trajectory through repeated experimental epiphyte removal of the logs and through different seasons. In this way, we separated the confounding effects of decay class and ongoing epixylic vegetation succession by creating new exposed dead wood surfaces upon each sampling event and for each decay class separately.

MATERIALS AND METHODS

Site Description

The study was conducted at the Long-term Research Station of Alpine Forest Ecosystems (102°53′–102°57′E, 31°14′–31°19′N, altitude 2458–4619 m) in Li County, Sichuan, southwestern China. The station is located in the transition zone from the Tibetan Plateau to the Sichuan Basin. The mean annual temperature and precipitation are approximately 2–4 °C and 850 mm, respectively. The seasonal soil freeze–thaw cycle begins in early November after the first snow fall, and the soil remains frozen until April of the following year. The soils include Cambisols and Primosols (Chang and others 2015).

Minjiang fir (*Abies faxoniana* Rehder & E. H. Wilson) is the dominant tree species (with an average tree age of 130 years) in the experimental forest site. The mass proportions of bark, sapwood and heartwood to the total (undecomposed) dry stem biomass are 24.4–25.1%, 51.3–65.6% and 10.9–23.6%, respectively (authors' unpublished data). The forest in the region is old-growth forest without clearly visible indications of human activities. In the target plots, the average proportion of Minjiang fir basal area of the total stand basal area is almost 80%, the proportion of *Sabina saltuaria* (Rehder & E. H. Wilson) W. C. Cheng & W. T. Wang is almost 20%, and the proportions of *Betula albosinensis* Burkill and *Larix mastersiana* Rehder & E. H. Wilson vary between 2 and 5% (Chang and others 2015). The extant pool of CWD in the site is 53 t ha⁻¹ (Xiao and others 2016).

Experimental Design

In August 2013, the treatments, including epixylic vegetation removal (ERM), were assigned to the permanent plot sized 100 m × 100 m in a Min-

jiang fir forest (31.23°N, 102.88°E, 3582 m a.s.l.). In the permanent plot, below a closed forest canopy, three replicate 25 m × 25 m subplots were positioned (Figure 1) for the present study.

Based on a system of five decay classes (Table 1), 90 logs of Minjiang fir (18 logs for each decay class, 120 cm in length and 35 ± 5 cm in diameter) were collected for the experiment from a primary Minjiang fir forest. The tree logs collected for the experiment had died naturally except for trees of DC I, which were cut fresh to ensure that the start of the decay process was included. Logs with large side branches and/or irregular shapes were avoided. All the logs were then transported to the incubation plot carefully with minimum damage to the bark and the underlying wood. Within each subplot below the closed canopy, a set of 30 logs (2 treatments × 3 replications × 5 DCs) were positioned on the soil surface 30 cm apart to ensure close contact with the soil and minimal interaction between logs (Figure 1). Three logs of each decay

class were selected for manual removal of the epixylic vegetation with the help of a knife. Each month, any newly established epixylic vegetation was removed manually. The other three logs per subplot and decay class served as controls.

Sampling and analysis

According to our previous studies and long-term sequential temperature observations (He and others 2015; Ni and others 2015), we divided the entire year into the snow-covered period, the snow thawing period, the early growing season and the late growing season. We began sampling on February 26, 2014 (snow-covered period), after 195 days of incubation, and then on April 23, 2014 (snow thawing period), August 20, 2014 (early growing season), and October 22, 2014 (late growing season). To characterize the temperature dynamics of each sampling season (SS), we calculated the mean daytime temperature (MDT) and mean night-time temperature (MNT) (Figure A2).

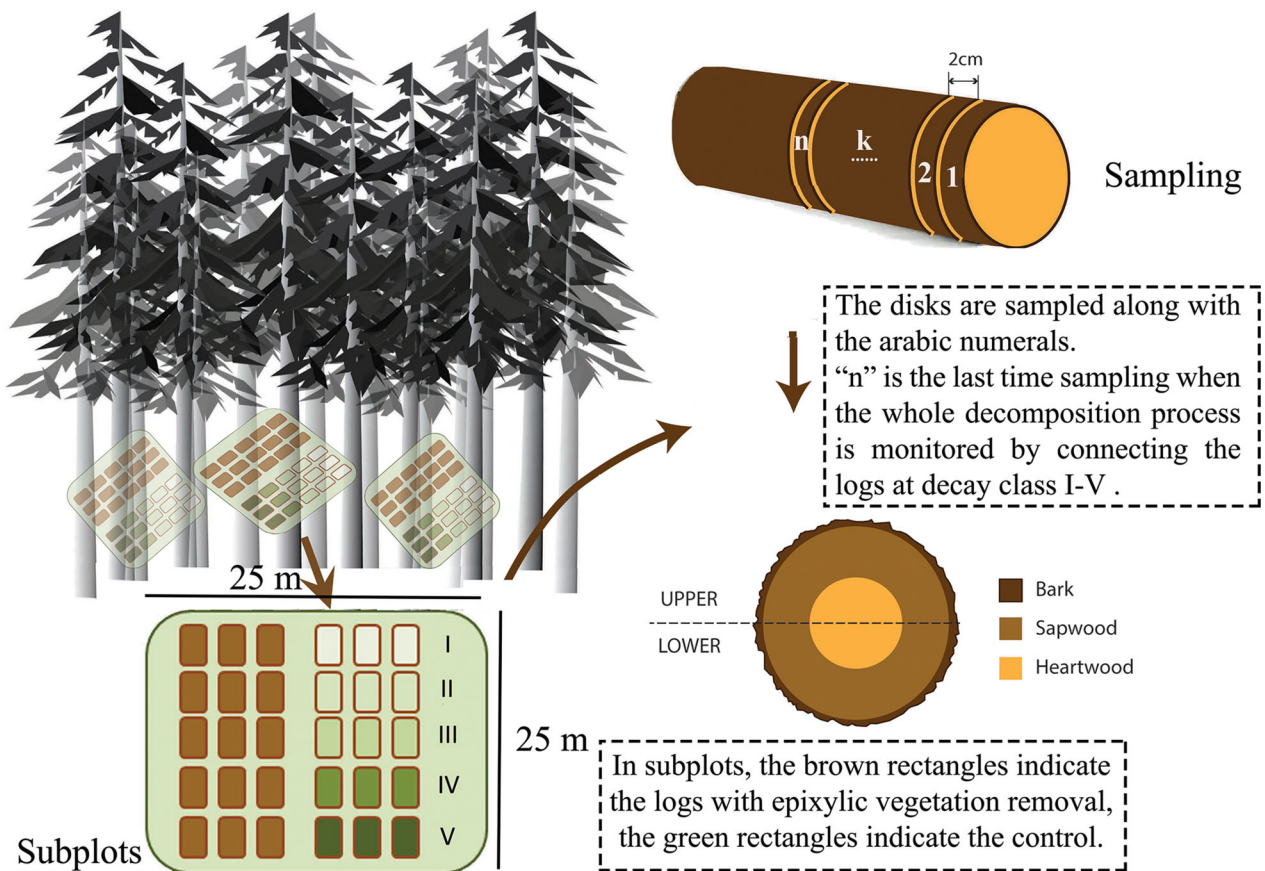


Figure 1. Experimental design and sampling of logs of *Abies faxoniana* for the wood decomposition experiment with log decay classes ranging from I (fresh dead wood) to V (very strongly decomposed wood) in an alpine fir forest on the eastern Qinghai–Tibetan Plateau. From each sampled disk, three cubes ($2 \times 2 \times 2$ cm³) were taken from each of five components: heartwood, sapwood in upper log part, sapwood in lower log part, bark on upper part of log and bark on lower part of log.

Table 1. The Features of Decaying Logs of DCs I–V in an Alpine Fir forest on the Eastern Qinghai–Tibetan Plateau

Decay class	Structural integrity	Epixylic vegetation colonization	Bark presence–absence
I	Sound, cambium still fresh	Little or no moss or other vegetation	Intact
II	Sound, cambium decayed	Sparse moss or other vegetation	Mostly intact
III	Maintained, heartwood mostly sound	Sparse moss or other vegetation	Detached or absent
IV	Heartwood rotten, oval in shape	Covered with moss or other vegetation	Mostly absent
V	Partial collapsed	Covered in thick moss-dominated vegetation	Absent

The air temperature was measured at 2-h intervals over the entire study period (August 15, 2013–October 22, 2014) using iButton DS1923-F5 Recorders (iButton DS1923-F5, Maxim/Dallas Semiconductor, Sunnyvale, CA, USA), which were placed in litterbags (nylon, 20 × 20 cm, 0.5 mm mesh size) hung on trees in a subplot in each plot to protect the recorders from direct sunlight and precipitation.

Data on microbial PLFA and moisture contents were collected in each sampling season, and data on pH were collected only in the snow-covered period and the early growing season. Three logs from each DC were chosen for the present study. For the logs from DCs I–III, one 2-cm thick disk was sawed out at the same end at each harvest (Figure 1), and then 3 cubes (2 × 2 × 2 cm³) were cut out from each of the five components (heartwood, sapwood in upper and lower part log, bark on upper and lower part of log). The upper and lower parts of the sapwood and bark were divided by an imaginary horizontal line halfway from top to bottom when the log was lying on the ground (Figure 1). For logs in advanced decayed stages (IV–V), adequate fragments of broadly similar volume were collected. With the use of sterilized sealing bags (12 cm × 15.5 cm), the subsamples of sapwood and bark from the upper and lower positions (Figure 2) were separated in different compartments of the bag, whereas the subsamples of heartwood were extracted from the center of the disk. All the subsamples were transported to the laboratory in an ice box within 24 h.

The log water content (g of water per 100 g fresh sample for each structural component) was measured by oven drying the samples at 60°C until constant mass was reached. The pH was measured by mixing wood powder and demineralized water (mass ratio 1:8). After 1 h of shaking, the supernatant was measured using a pH meter (PHS-25CW, Bante Instrument, Shanghai, PR China).

The samples for microbial PLFA extraction were immediately freeze-dried and stored at –70°C. The PLFA content was extracted using the method described by Bligh and Dyer (1959) and Wilkinson and others (2002). Converted PLFAs (fatty acid methyl esters (FAMES)) were separated, quantified and detected using a SHIMADZU gas chromatograph (GC) equipped with a mass spectrometer (QP2010-Ultra) and a GC column (Cat NO. 13623) and controlled by an operation system with reference to standards. FAMES standards were obtained from Supelco (Bacterial Acid Methyl Ester Mix, 47080-U; 37 Component Fatty Acid Methyl Ester Mix, CRM47885). Internal standard methyl nonadecanoate (C19:0) was obtained from Supelco (74208-1G). The PLFA biomarkers were used to determine the fungal population (18:2ω6c), the bacterial population (sum of 15:0, i15:0, a15:0, 16:0, 16:1ω5t, 16:1ω7c, 16:1ω9c, 17:0, a17:0, i17:0, cy17:0, 18:1ω7c, cy19:0) and the micro-eukaryote (Micro) population (sum of 18:3 and 20:4). The total microbial biomass was the sum of all PLFA signatures detected. The Gram⁺ bacterial population was the sum of i15:0, a15:0, i17:0 and a17:0. The Gram[–] bacterial population was the sum of 16:1ω7c, 16:1ω9c, cy17:0, 18:1ω7c and cy19:0.

Data Analysis

The total microbial, bacterial and fungal PLFA biomasses were calculated as the difference between treatment and control values, i.e., $\Delta = E^- - E^+$ (E^- , PLFA biomass after ERM; E^+ , PLFA biomass with epixylic vegetation, the control). For the control data, please see (Chang and others 2017).

The differences between the control group and the removal treatment group were evaluated using a paired-sample *t* test with an alpha level of 0.05.

To test the effects and interactions of ERM, log component (C), sampling season (SS), and DC, we employed a generalized linear regression for the

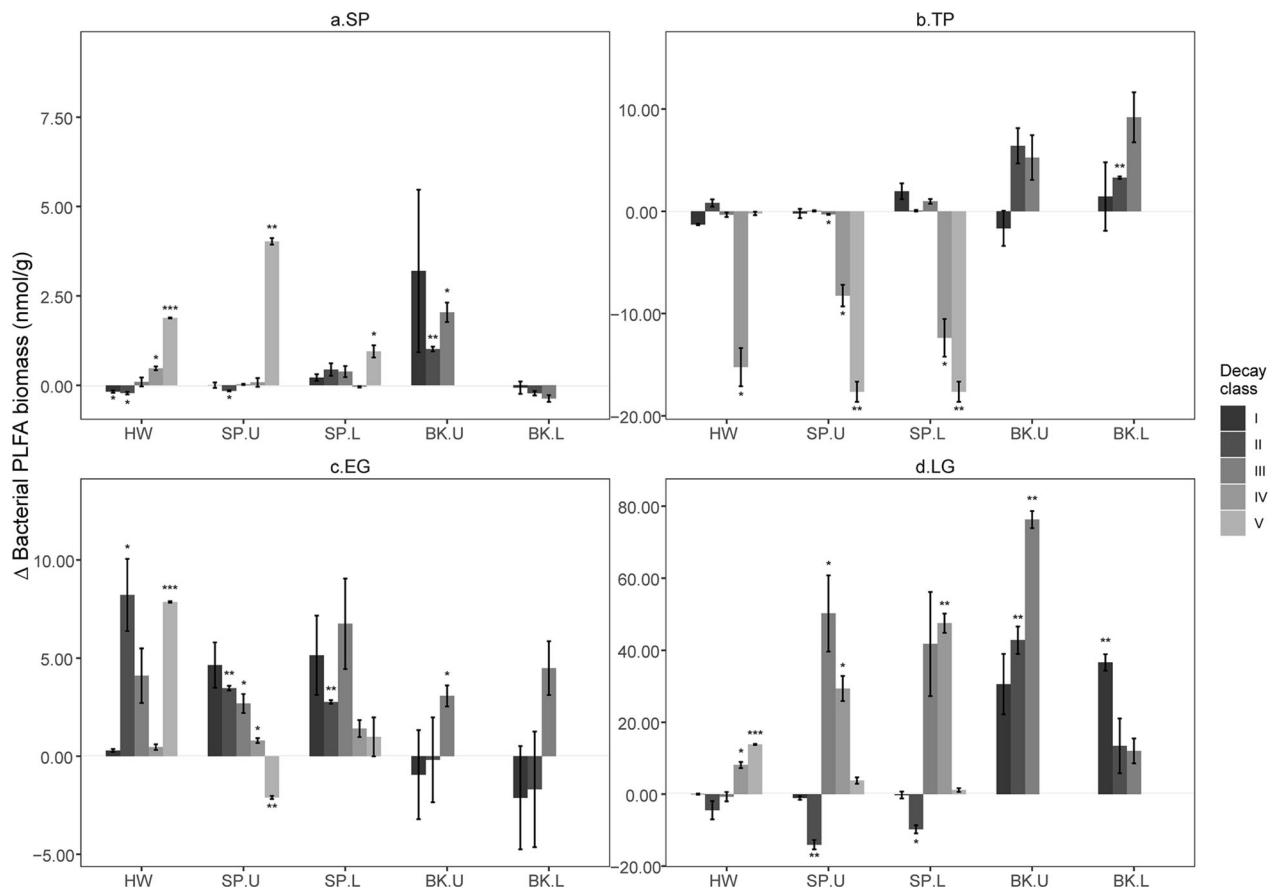


Figure 2. Changes in bacterial PLFA biomass in five components of logs of different decay classes affected by ERM in an alpine fir forest on the eastern Qinghai-Tibetan Plateau during the snow-covered period (SP), snow thawing period (TP), early growing season (EG) and late growing season (LG). Data represent the relative value, that is, $\Delta = E^- - E^+$ (E^- , PLFA biomass after ERM; E^+ , PLFA biomass with epixylic vegetation, the control). For the underlying control data, see Chang and others (2017). Error bars represent the standard errors of the means ($n = 3$). Asterisks indicate significance levels of differences of mean values (E^-) compared to zero change (E^+): $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. HW: heartwood; SP.U: sapwood in upper log part; SP.L: sapwood in lower log part; BK.U: bark on upper part of log; BK.L: bark on lower part of log. Note: To show each bar clearly, the scales differ within the subplots as the effect of ERM increases over time.

PLFA total microbial biomass, bacterial biomass, fungal biomass and α -diversity. Moreover, we constructed a predictive model to identify differences in the Sørensen similarity (β -diversity) for the microbial composition (based on PLFA signature) under the null hypothesis of no interaction effects and the equal influence of each factor (ERM, C, SS, DC). As a result, we assumed that the lowest value in the Sørensen similarity index would be the combination of the factors for SS and DC in the control or removal treatment. Thereafter, we also expected a consistent influence of ERM on the Sørensen similarity index. We combined the predictive model and the actual results to test the effects of different combinations of drivers, where strong deviations from expectations were interpreted as (non-additive) interactions.

We calculated the α - and β -diversity of the microbial community based on the number of individual PLFA occurrences as follows: α -diversity represents the PLFA signature richness in each decay class and was expressed as the averaged occurrence number of individual PLFA signatures per log in each decay class; β -diversity represents (Wardle and others 2015) the dissimilarity in PLFA signature composition between logs. The computer programme EstimateS 9 (Colwell 2005) was used to calculate the Sørensen similarity index (Cs):

$$Cs = 2j/(a + b) \quad (1)$$

where j represents the number of PLFA signatures occurring in both logs, a represents the number of PLFA signatures in log A and b represents the number of PLFA signatures in log B. The Cs value is

designed to equal 1 in cases of complete similarity and 0 in cases of complete dissimilarity.

Before the analysis of the paired-sample t-test and generalized linear model, the data that did not meet the requirement of standard normal distribution were transformed (natural log, square root or sin) to improve the normality and homogeneity of variance. Statistical significance was determined at the level of 0.05. Data for each PLFA monomer were square root transformed prior to a redundancy analysis (RDA) to test for the linkages of PLFA composition with gradients of substrate moisture and pH. The RDAs were conducted using Canoco for Windows (version 5.0).

RESULTS

Microbial Biomass

The effects of ERM on the total microbial biomass and bacterial biomass strengthened over time but varied with the components in different sampling seasons (Figures 2, A3). Compared with the other two log components, total microbial biomass and bacterial biomass in bark were most affected by ERM (Table 2). Larger changes in total microbial

biomass and bacterial biomass were observed in bark subjected to ERM throughout the sampling period except on the lower side of the log in the snow-covered period and early growing season. Both biomasses were significantly depressed in the snow thawing period and enhanced in the early growing season in heartwood and sapwood after ERM. The difference within decay classes between heartwood and sapwood also had a less pronounced influence on total and bacterial biomass, with DCs IV/V always responding strongly to the ERM and DCs I/II showing only a strong response in the early growing season and late growing season (Table 2, Figure 2). Compared with the lower part of the log, the upper part of the log showed a stronger response to the ERM.

ERM also had a pronounced effect ($P < 0.05$) on fungal biomass of heartwood and bark (Table 2). The effect was concentrated mainly during the snow thawing period and the early growing season (Figure 3), when fungi also tend to reach a high proportion, especially in the snow thawing period (Figure 4). Fungal biomass was enhanced after ERM for all log components in the early growing season and for heartwood and bark on the lower

Table 2. Results of a Generalized Linear Model of Removal, Sampling Season, and Decay Class on Total Microbial Biomass, Bacterial Biomass, Fungal Biomass and α -Diversity In Decaying Logs in an Alpine Fir Forest on the Eastern Qinghai–Tibetan Plateau

	Df	F			
		Total microbial biomass	Bacterial biomass	Fungal biomass	α -diversity
<i>Heartwood</i>					
Removal	1	4.850*	3.324	15.719***	0.089
Sampling season (SS)	3	45.833***	47.860***	49.989***	147.345***
Decay class (DC)	2	16.552***	19.425***	7.140*	6.059*
Removal \times SS	3	62.461***	57.112***	56.778***	6.661
Removal \times DC	2	3.233	4.104	2.471	7.875*
Removal \times SS \times DC	6	32.378***	39.937***	37.972***	5.625
<i>Sapwood</i>					
Removal	1	18.665***	37.432***	0.617	5.287*
Sampling season (SS)	3	117.742***	245.289***	127.792***	339.128***
Decay class (DC)	2	21.591***	46.244***	18.599***	27.915***
Removal \times SS	3	22.337***	46.235***	137.487***	21.713***
Removal \times DC	2	46.896***	96.198***	16.913***	20.232***
Removal \times SS \times DC	6	136.358***	273.523***	32.019***	30.256***
<i>Bark</i>					
Removal	1	107.487***	158.271***	13.614***	10.105**
Sampling season (SS)	3	1107.903***	1752.632***	218.980***	377.368***
Decay class (DC)	2	3.533	6.250*	22.872***	31.500***
Removal \times SS	3	208.595***	325.461***	1.623	8.526*
Removal \times DC	2	6.832*	11.314**	6.135*	42.711***
Removal \times SS \times DC	6	7.065	182.418***	12.823*	62.763***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

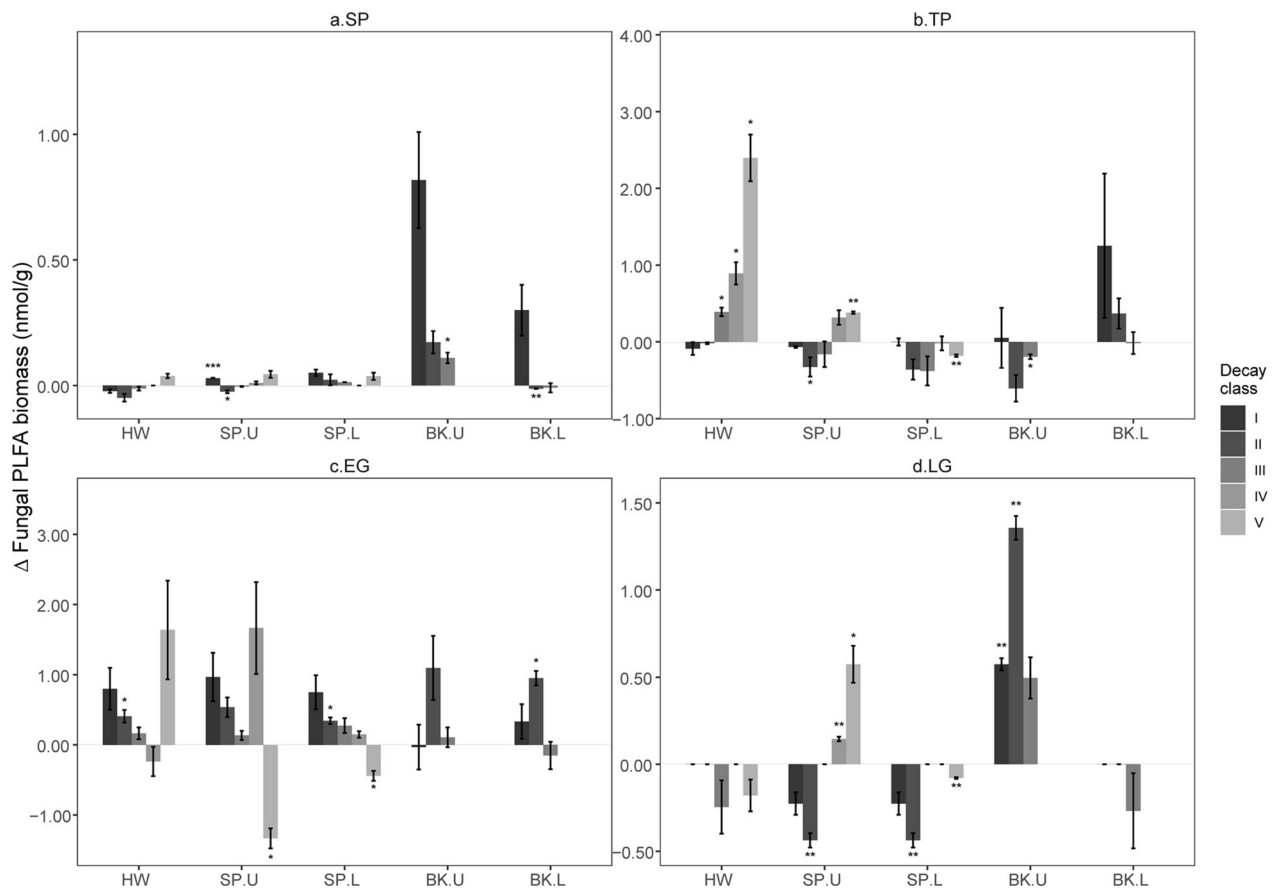


Figure 3. Changes in fungal PLFA biomass in five components of logs of different decay classes as affected by ERM in an alpine fir forest on the eastern Qinghai-Tibetan Plateau during the snow-covered period (SP), snow thawing period (TP), early growing season (EG) and late growing season (LG). Data represent the relative value, that is, $\Delta = E^- - E^+$ (E^- , PLFA biomass after ERM; E^+ , PLFA biomass with epixylic vegetation, the control). For the underlying control data, see Chang and others (2017). Error bars represent the standard errors of the means ($n = 3$). Asterisks indicate significance levels of differences of mean values (E^-) compared to zero change (E^+): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. HW: heartwood; SP.U: sapwood in upper log part; SP.L: sapwood in lower log part; BK.U: bark on upper part of log; BK.L: bark on lower part of log. Note: To show each bar clearly, the scales differ within the subplots as ERM had a different effect on the fungal biomass across sampling seasons.

log side in the snow thawing season, while fungal biomass in sapwood bark on the upper side was decreased.

Microbial Community Structure

The microbial α -diversity and Sørensen similarity (Eq. 1) showed a pronounced response to ERM (Tables 2, 3, A1). The response of α -diversity to ERM varied among the log components, with only sapwood and bark showing significant responses ($P < 0.05$). Sampling seasons showed a stronger effect than decay classes (Table 2). The interactions of ERM response with decay classes and sampling seasons were generally much stronger than the main effect of ERM on α -diversity.

The inconsistency in the results for the Sørensen similarity patterns indicated that interactions occurred between ERM and sampling seasons, and decay classes (Table 3). Originally, we had expected a consistent response (decrease, increase or invariable) for the Sørensen similarity values. However, strong differences among log components were observed, including an obvious decrease in sapwood and increase in bark. The relationship between ERM and Sørensen similarity also varied with the sampling seasons and decay classes. In heartwood and sapwood, the relationship was most strongly affected by sampling seasons, whereas the combination of ERM, sampling seasons and decay classes had the strongest effect on the Sørensen similarity. A notable increase in Sørensen value was observed in the bark and

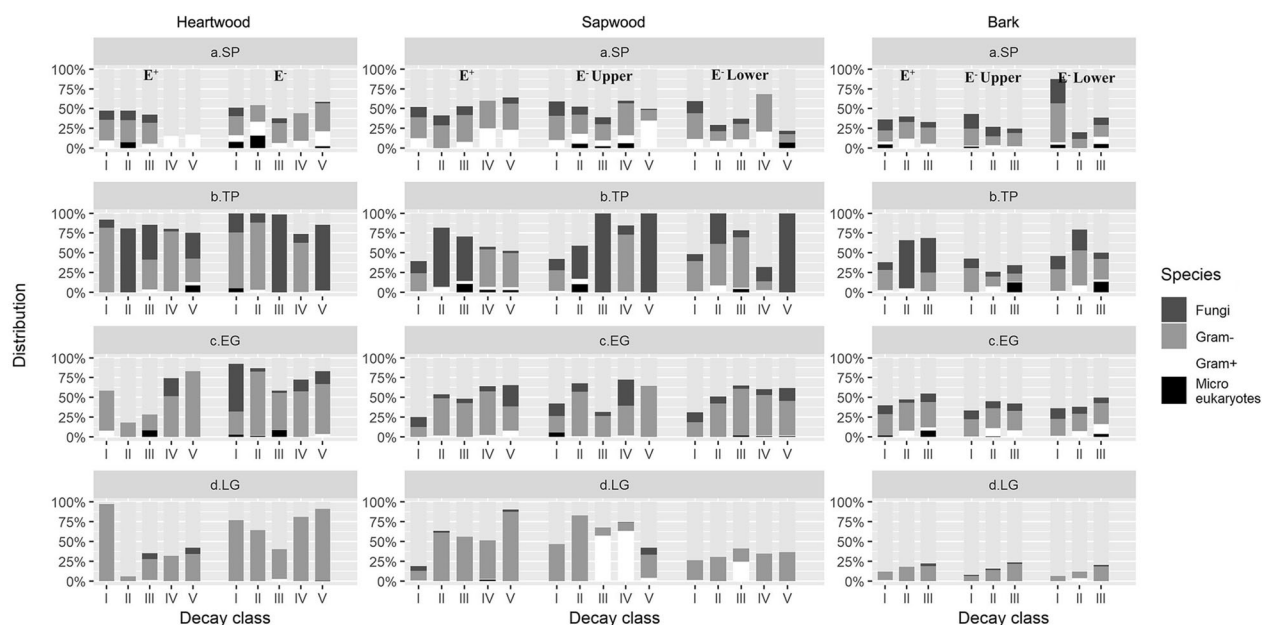


Figure 4. The distribution of microbial higher taxa in logs of different decay classes as affected by ERM in an alpine fir forest on the eastern Qinghai–Tibetan Plateau during the snow-covered period (SP), snow thawing period (TP), early growing season (EG) and late growing season (LG). The PLFA biomass of each taxonomic group is given as a cumulative percentage of total microbial PLFA biomass; here, we include only the four main taxa in decaying logs, that is, micro-eukaryotes, fungi, Gram⁺ bacteria and Gram[−] bacteria. E⁺, control (log without epixylic species removal); E[−] Upper, upper part of log after ERM; E[−] Lower, lower part of log after ERM.

heartwood samples across a combination of decay classes and sampling seasons (I/II + EG, II/III + TP, IV/V + SP), whereas a decrease was observed in the sapwood samples (I/II + LG, III/V + TP).

ERM evidently altered the microbial community composition in the logs both at the functional group level (Figures 4, A4) and the PLFA signature level (Figure 5). The ratio of fungi to bacteria was most strongly affected in bark; moreover, Gram[−] bacteria were more affected than Gram⁺ bacteria in all log components. Fungi and Gram[−] bacteria dominated the microbial community in the control group of heartwood and sapwood, whereas a richer composition in microbial taxa other than the four main taxa (as implied by the large empty sections below the 100% level in Figure 4) was detected in bark. The total percentage of fungal and Gram[−] bacteria in heartwood and upper side sapwood both increased after ERM (Figure 4). The total percentage of fungal and Gram[−] bacteria in upper side bark decreased after ERM except in the late growing season. At the PLFA signature level (Figure 5), the microbial community composition in sapwood and bark of the upper side in the late decay class was more affected by ERM, especially in the early growing season.

Relationships Between ERM and Moisture Content, pH and Microbial Community

A significant effect of ERM on moisture content and pH was observed, and the seasonal fluctuations in moisture content were strengthened, while those in pH weakened after ERM (Figure 6, Table 4), especially in the log bark. Moreover, the RDA results indicated that changes in moisture content and pH significantly controlled the distribution of the microbial community (Figure 5). The moisture content in the upper part of the log was more affected by ERM than that in the lower part during the study period (Figure 6).

DISCUSSION

Strong effects of ERM on the microbial community were observed, with log components and sampling seasons all playing significant roles and decay class showing a less pronounced effect, which partially supported our hypotheses. In our study, fungi and Gram[−] bacterial dominated and responded strongly to ERM in the decaying logs. ERM strongly altered the biomass and composition of microbial taxa in bark and showed less profound but more complex effects on the heartwood and sapwood inside.

Table 3. Sørensen Similarity Patterns Model for the Microbial Communities in Decaying Logs as a Function of ERM (Here, We Used R in the Predictive Model), Combined with Log Component (C, Heartwood, Sapwood, Bark), Decay Class (DC, I–V) and Sampling Season (SS)

I		II				III			
SP	TP	EG	LG	SP	TP	EG	LG	SP	TP
(a) Predictive model									
I									
SP									
TP	R + SS								
EG	R + SS	R + SS							
LG	R + SS	R + SS							
II									
SP	R + DC	R + SS + DC	R + SS + DC	R + SS + DC					
TP	R + SS + DC	R + DC	R + SS + DC	R + SS + DC	R + SS				
EG	R + SS + DC	R + SS + DC	R + DC	R + SS + DC	R + SS	R + SS			
LG	R + SS + DC	R + SS + DC	R + DC	R + DC	R + SS	R + SS			
III									
SP	R + DC	R + SS + DC	R + SS + DC	R + DC	R + SS + DC	R + SS + DC	R + SS + DC	R + SS + DC	
TP	R + SS + DC	R + DC	R + SS + DC	R + SS + DC	R + SS + DC	R + DC	R + SS + DC	R + SS + DC	R + SS
EG	R + SS + DC	R + SS + DC	R + DC	R + SS + DC	R + SS + DC	R + DC	R + SS + DC	R + SS + DC	R + SS
LG	R + SS + DC	R + SS + DC	R + DC	R + DC	R + SS + DC	R + SS + DC	R + DC	R + SS + DC	R + SS
Heartwood	I	II	III	IV	V				
SP	SP	EG	LG	SP	TP	EG	LG	SP	TP
TP	TP	TP	TP	TP	TP	TP	TP	TP	TP
EG	EG	EG	EG	EG	EG	EG	EG	EG	EG
LG	LG	LG	LG	LG	LG	LG	LG	LG	LG
(b) Predictive model combined with actual results									
I									
SP	-								
TP	0.67	-							
EG	0.70	0.86	-						
LG	0.39	0.40	0.45	-					
II									
SP	0.71	0.55	0.59	0.36	-				
TP	0.66	0.76	0.71	0.43	0.55	-			
EG	0.63	0.66	0.64	0.33	0.51	0.71	-		
LG	0.50	0.50	0.50	0.41	0.45	0.47	0.41	-	
III									
SP	0.77	0.71	0.71	0.38	0.65	0.68	0.62	0.50	-
TP	0.49	0.60	0.60	0.29	0.34	0.64	0.48	0.26	0.49
EG	0.64	0.60	0.61	0.35	0.59	0.63	0.54	0.42	0.60
LG	0.47	0.42	0.44	0.33	0.45	0.45	0.34	0.39	0.44

Table 3. continued

Heartwood	I				II				III				IV				V			
	SP	TP	EG	LG	SP	TP	EG	LG	SP	TP	EG	LG	SP	TP	EG	LG	SP	TP	EG	LG
IV																				
SP	0.82	0.64	0.66	0.43	0.79	0.67	0.61	0.56	0.76	0.29	0.67	0.49	-							
TP	0.74	0.64	0.68	0.52	0.62	0.75	0.65	0.58	0.68	0.57	0.65	0.50	0.69	-						
EG	0.83	0.81	0.85	0.50	0.69	0.70	0.67	0.57	0.83	0.60	0.66	0.47	0.80	0.73	-					
LG	0.62	0.55	0.61	0.55	0.58	0.52	0.50	0.62	0.63	0.37	0.51	0.48	0.66	0.70	0.74	-				
V																				
SP	0.80	0.66	0.68	0.42	0.72	0.66	0.64	0.54	0.75	0.48	0.66	0.46	0.81	0.81	0.79	0.68	-			
TP	0.66	0.57	0.65	0.43	0.60	0.80	0.59	0.35	0.62	0.72	0.56	0.40	0.56	0.75	0.70	0.52	0.66	-		
EG	0.77	0.64	0.68	0.47	0.66	0.80	0.65	0.49	0.68	0.57	0.67	0.49	0.76	0.87	0.73	0.59	0.84	0.80	-	
LG	0.57	0.51	0.54	0.49	0.50	0.49	0.46	0.57	0.57	0.32	0.45	0.43	0.59	0.64	0.66	0.74	0.61	0.43	0.52	-
Sapwood																				
I																				
SP	-																			
TP	0.77	-																		
EG	0.71	0.77	-																	
LG	0.67	0.62	0.64	-																
II																				
SP	0.85	0.72	0.66	0.61	-															
TP	0.60	0.69	0.57	0.43	0.55	-														
EG	0.76	0.80	0.77	0.65	0.71	0.62	-													
LG	0.62	0.57	0.58	0.64	0.60	0.36	0.56	-												
III																				
SP	0.85	0.78	0.70	0.62	0.82	0.60	0.75	0.60	-											
TP	0.54	0.62	0.57	0.37	0.53	0.56	0.57	0.36	0.56	-										
EG	0.72	0.74	0.75	0.60	0.70	0.56	0.75	0.58	0.73	0.56	-									
LG	0.71	0.61	0.63	0.64	0.64	0.41	0.61	0.67	0.63	0.39	0.58	-								
IV																				
SP	0.68	0.57	0.55	0.53	0.67	0.42	0.57	0.60	0.66	0.39	0.60	0.61	-							
TP	0.76	0.84	0.74	0.61	0.72	0.72	0.80	0.50	0.77	0.61	0.74	0.56	0.55	-						
EG	0.82	0.78	0.73	0.61	0.77	0.61	0.76	0.58	0.80	0.56	0.73	0.61	0.61	0.78	-					
LG	0.74	0.65	0.67	0.64	0.67	0.46	0.66	0.73	0.67	0.43	0.64	0.73	0.62	0.61	0.67	-				

Table 3. continued

Sapwood	II					III					IV					V				
	SP	TP	EG	LG	SP	TP	EG	LG	SP	TP	EG	LG	SP	TP	EG	LG	SP	TP	EG	LG
<i>V</i>																				
SP	0.86	0.77	0.75	0.67	0.79	0.61	0.77	0.64	0.81	0.53	0.76	0.68	0.67	0.76	0.82	0.74	-	-	-	-
TP	0.53	0.59	0.58	0.37	0.51	0.63	0.57	0.38	0.54	0.72	0.59	0.40	0.41	0.60	0.54	0.46	0.54	-	-	-
EG	0.76	0.70	0.69	0.62	0.75	0.49	0.71	0.63	0.76	0.49	0.72	0.63	0.63	0.70	0.76	0.67	0.78	0.46	-	-
LG	0.74	0.66	0.66	0.61	0.69	0.46	0.66	0.67	0.70	0.44	0.65	0.67	0.62	0.61	0.70	0.71	0.76	0.46	0.71	-
<i>Bark</i>																				
	II					III					IV					V				
	SP	TP	EG	LG	SP	TP	EG	LG	SP	TP	EG	LG	SP	TP	EG	LG	SP	TP	EG	LG
<i>I</i>																				
SP	-																			
TP	0.84	-																		
EG	0.86	0.87			-															
LG	0.76	0.71			0.76	-														
<i>II</i>																				
SP	0.86	0.79			0.82	0.74														
TP	0.84	0.84			0.84	0.68				-										
EG	0.87	0.83			0.86	0.71				0.86										
LG	0.71	0.69			0.73	0.74				0.70	0.73									
<i>III</i>																				
SP	0.93	0.85			0.86	0.76				0.86	0.89		0.72		-					
TP	0.89	0.83			0.85	0.69				0.85	0.84		0.69		0.85					
EG	0.85	0.81			0.81	0.69				0.85	0.85		0.68		0.88					
LG	0.84	0.78			0.87	0.85				0.74	0.80		0.74		0.84		0.82	-	0.75	-

(a) Predictive model. Abbreviations in bold represent the factors that affect the similarity of microbial composition between logs.

(b) Predictive model combined with actual results.

SSs SP, snow-covered period; TP snow thawing period; EG early growing season; LG late growing season.

Sørensen similarity values indicate the comparisons between two groups of logs (mean) after ERM. Values in bold and italic font indicate 10% higher and lower values than the control, respectively.

Table 4. pH of Heartwood, Sapwood and Bark of Decaying Logs with Different DCs (I–V) as Affected by ERM in an Alpine Fir Forest in the Eastern Qinghai–Tibetan Plateau During Snow-Covered Period (SP) and Early Growing Season (EG)

Components	Sampling season	Decay class	Control	Removal	
				Upper part	Lower part
Heartwood	SP	I	6.65 (0.09)	6.00 (0.02)	
		II	5.60 (0.22)a	6.37 (0.04)b	
		III	4.89 (0.03)a	5.71 (0.09)b	
		IV	4.32 (0.02)	4.35 (0.02)	
		V	3.77 (0.08)	3.66 (0.21)	
	EG	I	6.57 (0.01)	5.79 (0.06)	
		II	6.20 (0.11)	6.39 (0.07)	
		III	5.02 (0.07)a	5.98 (0.04)b	
		IV	4.66 (0.05)	4.48 (0.01)	
		V	3.89 (0.02)a	4.04 (0.02)b	
Sapwood	SP	I	5.84 (0.65)	5.57 (0.03)	5.39 (0.31)
		II	5.86 (0.27)	5.73 (0.13)	6.11 (0.37)
		III	5.13 (0.18)	5.20 (0.63)	5.24 (0.39)
		IV	4.67 (0.03)a	4.30 (0.1)b	4.21 (0.02)b
		V	4.26 (0.08)	4.15 (0.02)	4.15 (0.05)
	EG	I	5.75 (0.52)	6.41 (0.1)	6.62 (0.08)
		II	5.86 (0.17)a	6.28 (0.06)a	6.39 (0.08)b
		III	4.59 (0.02)a	5.85 (0.05)b	4.78 (0.25)b
		IV	4.32 (0.01)	4.33 (0.03)	4.30 (0.01)
		V	3.59 (0.03)a	4.34 (0.03)b	3.89 (0.01)b
Bark	SP	I	5.01 (0.13)a	5.08 (0.04)a	5.44 (0.02)b
		II	5.56 (0.14)	5.39 (0.06)	5.59 (0.07)
		III	5.21 (0.08)a	5.61 (0.11)b	5.79 (0.09)b
	EG	I	5.40 (0.06)a	5.38 (0.14)a	4.93 (0)b
		II	5.83 (0.06)a	5.56 (0.03)b	5.11 (0.06)b
		III	5.84 (0.01)a	5.06 (0.04)b	5.78 (0.07)a

Different lowercase letters indicate significant differences between control and ERM treatment at the 0.05 level. The data are presented as the mean (SE, $n = 3$).

Moreover, the heartwood and sapwood in the upper part of the log responded more strongly to ERM than in the lower part. The changes in the moisture content and pH value resulting from ERM were closely related to the dynamic patterns of the microbial community in the logs in the alpine forest. Our study highlighted the role of epixylic vegetation in regulating the microbial community, possibly via altering the microclimate, which may greatly influence the forest ecosystem carbon and nutrient cycles.

Effects of ERM and Log Components

A pronounced and direct influence of ERM on the bark microbial biomass and distribution was observed. Because the bark of *A. faxoniana* initially comprises one-quarter of the total (undecomposed) log biomass (see Materials and Methods), this finding could have important implications for dead wood carbon turnover. It is likely that epixylic

vegetation plays an important role in regulating the access of a vast array of microbial species to bark and in regulating enzyme activity functions by affecting nutrient availability, secondary chemistry and hydrothermal control (Rundel 1978; Hagemann and others 2010). Nutrient competition, especially in the log surface layer between microbial organisms and epixylic vegetation, has so far been ignored; however, this competition may also have contributed to the changes in microbial community composition with ERM. Additionally, the diverse herbivorous and other invertebrates attracted by the epixylic vegetation have the potential to attract a number of new microorganisms to bark and the wood inside it (Davey and others 2012) and may thus affect the decay process. Previous studies (Dossa and others 2016; Ulyshen and others 2016) demonstrated that the presence and absence of bark can enhance or inhibit the decay rate of the wood by directly affecting

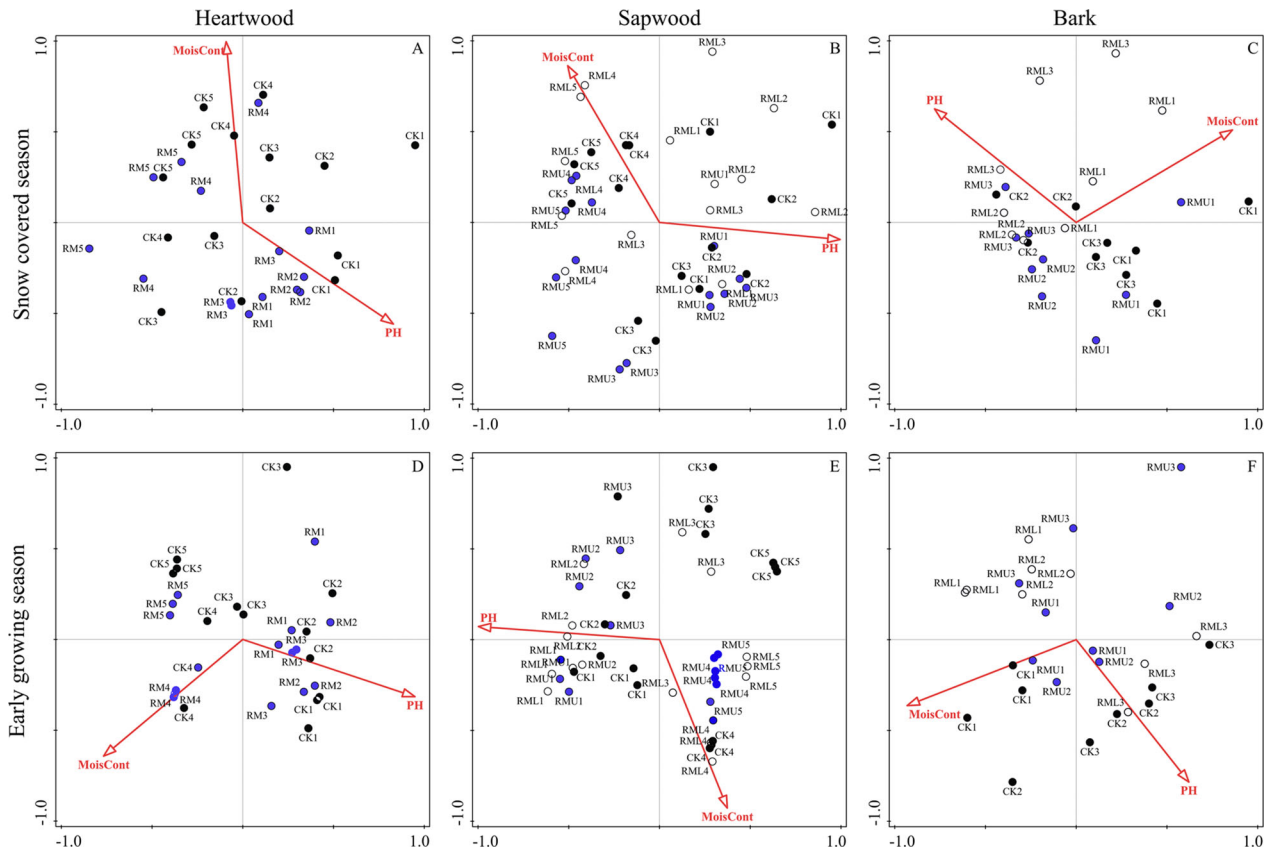


Figure 5. RDA plot of PLFA profiles for decaying heartwood, sapwood and bark collected during the snow-covered period and early growing season in the alpine fir forest on the eastern Qinghai-Tibetan Plateau. MoisCont, moisture content of heartwood, sapwood and bark. CK, control (E^+ , filled dark circles); RMU, upper part of log after ERM (E^- , filled blue circles); RML, lower part of log after ERM (E^- , open circles); Arabic numerals (1–5), DCs (Color figure online).

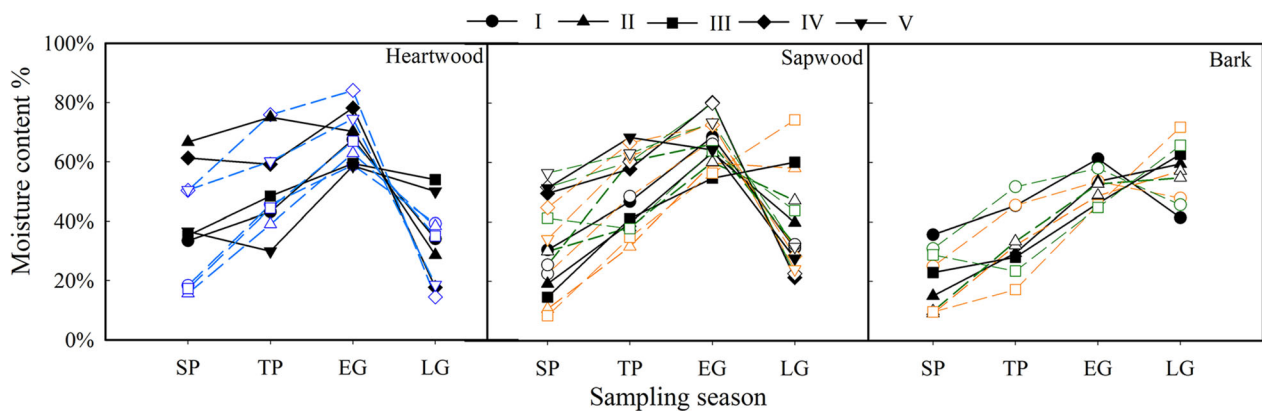


Figure 6. Moisture content of log components as affected by ERM from logs of different decay classes during the snow-covered period (SP), snow thawing period (TP), early growing season (EG) and late growing season (LG) in an alpine fir forest on the eastern Qinghai-Tibetan Plateau. DCs: I, circles; II, triangles; III, squares; IV, diamonds; V, upside-down triangles. Filled black symbols represent logs with control treatments; open symbols represent the logs with ERM. Different colors represent the five log components, that is, heartwood (blue), upper (orange) and lower parts (green) of sapwood and bark (Color figure online).

organisms involved in decomposition, but these studies overlooked the indirect path mediated by epixylic vegetation. Clearly, future study should

consider separating the effects of epixylic vegetation on the microbial community and activity. Compared with bark, the limited nutrient concen-

trations, gas diffusion and access of exogenous microorganisms in the inside log (heartwood and sapwood) (Ulyshen and others 2016; Dossa and others 2018) may all contribute to a higher sensitivity of the microbial community to even small changes in the environment, including changes due to ERM.

CWD microclimate was equally stable and humid as the forest floor in a previous study (Haughian and Frego 2017a), but that study did not differentiate the upper and lower parts of the log. Theoretically, compared with the upper part of the log, the lower part should receive a more stable hydrothermal supply from the ground (Graham 1925; Söderström 1988). Additionally, the convex upper surface of the log should be subjected to stronger evaporation. This effect explains why, in our study, the absence of epixylic vegetation growing on the lower side of the log caused a negligible response of the microbial community, whereas its absence on the upper side of the log greatly facilitated the stability of the microbial community.

Effects of ERM and Sampling Seasons, Decay Classes and Interaction of Sampling Seasons \times Decay Classes

Based on the Sørensen similarity model, our null hypothesis predicted that different drivers would have similar effect sizes as the ERM treatment and no interactive effect with ERM treatment on the microbial community composition in a pairwise comparison of the response of the different groups of logs. The experimental results presented strong deviations from the predicted variations through interactions of sampling seasons and decay classes with the removal treatment. Sampling season had a stronger effect than decay class on the ERM response. Moreover, the responses also differed in strength based on the combination of sampling seasons \times decay classes, and these interactions were positive, neutral or negative.

Epixylic vegetation is of functional importance for regulating microclimate, substrate nutrient regimes and biodiversity in decaying logs during different seasons. The absence of vegetation in different seasons would alter the pathway of the carbon cycle via influencing the microbial community. In the snow thawing season, the frost-resistant epixylic vegetation (mainly moss) (Lang and others 2012) could provide important habitats for arthropods and other invertebrate fauna, which may represent major microbial carriers after a harsh winter and likely contribute to the rapid restoration of microbial biomass. During the

growing season, the presence of epixylic vegetation growing on logs can reduce the erosion caused by rainfall and, in parallel, increase the concentration of secondary metabolites (Mues 2000; Bond-Lamberty and Gower 2007), some of which are inhibitory to microbial organisms and decrease the substrate pH. Previous studies (Coxson 1991; Mitchell and others 2005) also showed that a large quantity of organic compounds (such as monosaccharides and polyhydric alcohols) are released from epixylic vegetation in wet weather after drought. In dry seasons, the epixylic bryophytes also help to intercept a significant quantity of inorganic mineral nutrients (Rieley and others 1979; Nadkarni 1984, 1986; Benzing 1998), which are sparsely available for microorganisms feeding on woody debris. However, whether the functions are beneficial or harmful to the microorganisms may depend on the biomass and functional composition of epixylic vegetation growing on the decaying logs. The epixylic vegetation composition and succession on logs is closely associated with decay classes, though this association is probably not causal (Hubbell 2001; Haughian and Frego 2017b) but, rather, indirectly connected via the time available for colonization and the time for log decomposition.

In our study, the early and late growing seasons showed the most pronounced effect on ERM response. The early decay classes showed a positive ERM response in the early growing season and a negative response in late growing season. This pattern likely occurs because the epixylic vegetation (mainly facultative epiphytes) (Söderström 1988) growing on the logs is sparse and stochastic. Their composition can be easily disturbed by the changes in the seasonal environment. All these interactive effects can indirectly contribute to the differentiated response to the ERM (Zotz 2016). Conversely, epixylic vegetation on logs in late decay classes consists mainly of epigeous species (Söderström 1988) with thick cover, usually creating a low-temperature and high-humidity microclimate (Hagemann and others 2010) during the whole growing season. Moreover, the ground flora on logs of the late decay classes can produce a considerable amount of complex aromatic secondary metabolites with certain products presenting antibiotic properties (depsides, depsidones, diphenyl ethers, pulvinic acid derivatives, terpenes and other compounds) (Elix and Stocker-Wörgötter 1996) that greatly inhibit microbial biomass and activity. Vigorous growth of epixylic vegetation and boosted metabolism in the growing seasons may promote the interaction between sampling seasons and decay classes. Additional study will be neces-

sary to clarify the reasons for the association between sampling seasons and decay classes subject to epixylic vegetation cover.

Relationships Among ERM, Moisture and pH and the Microbial Community

Previous studies have found that epiphytic vegetation can enhance the moisture content and carbon concentration in bark (Shorohova and others 2016; Romashkin and others 2018). Our study further demonstrated that the cover of epixylic vegetation can alter both the microclimate of bark and the heartwood and sapwood inside it, henceforth affecting the microbial community on the decaying logs and thereby influencing the decomposition process. Fungi and Gram[−] bacteria dominated the decaying logs, especially in the wood, and were affected the most strongly by the absence of epixylic vegetation (Figure 4). This finding may be explained by the altered moisture content and pH (Klavina and others 2015; Shorohova and others 2016; Tláškal and others 2017), whereas the abundance of fungi and Gram[−] bacteria may increase the decay of the underlying wood. Similarly, another study also found that under the anaerobic conditions caused by high moisture content, basidiomycete activity is depressed and soft-rot fungi (Ascomycetes) and bacteria can become the dominant wood decomposition agents, which results in much slower decomposition rates (Stokland and others 2012). In the present study, the log moisture content after ERM showed greater seasonal fluctuations and became much drier in the winter and wetter in summer. In particular, bryophytes may have driven this removal response, as they are the predominant component of epixylic vegetation and can efficiently absorb water from precipitation and fog due to their poikilohydric water regime (Cornelissen and others 2007). They can also retain water rather well due to the aggregation of individual shoots into mats or cushions (Elumeeva and others 2011). Furthermore, the extensive foliage of the epixylic vegetation may provide protection from evaporation, rainfall erosion and nutrient leaching, especially in summer (Bond-Lamberty and Gower 2007).

In the present study, a lower pH value was detected during the snow-covered period than that during the early growing season of natural-growing epixylic vegetations on logs. In contrast to the moisture content, the seasonal fluctuation of the bark pH was reduced with ERM. There are three

possible reasons for the fluctuating pH in the control bark. First, the interception and accumulation of fallen litter and invertebrate corpses and fecal deposits by epixylic vegetation in the late growing season could result in more acidic conditions in the later decomposition phase of this fast decomposing material, that is, the snow-covered period (Ni and others 2015). Second, the vigorous growth of epixylic vegetation in the early growing season can produce more nitrogen, predominately in the form of ammonium, thus significantly elevating the bark pH. Third, the secondary metabolites produced by bryophytes contain a large proportion of organic acids, including depsides, pulvinic acid derivatives, terpenes and other compounds (Elix and Stocker-Wörgötter 1996; Frahm 2004), and these metabolites are also likely to be released or leached from the epixylic vegetation. Thus, pH functions more than as a predictor of the decay class of CWD (Zuo and others 2014) because it is both influenced by epixylic vegetation in one or more of the above ways and regulates biochemical and physiological processes (Zhao and others 2011; Gonzalez-Polo and others 2013), thereby acting as an environmental filter on the assembly of microbial communities (Hoppe and others 2015; Tláškal and others 2017). However, the mechanisms by which ERM influences microbial activity and related enzymes remain to be further studied.

CONCLUSIONS

This study has revealed significant and complex effects of epixylic plants on the microbial community of logs, which is likely to feed back to the decomposition rate and thereby biochemical cycling. The three log components, positions (upper/lower part) and decay classes during different sampling seasons all influenced the effect of ERM on the microbial community. However, the log components and sampling seasons had the strongest effect on ERM response; the response to ERM became progressively stronger from winter until late growing season. Furthermore, variations within components, positions and decay classes all lead to the increasing complexity of the response of the microbial community. Additional studies will be necessary to clarify how these interactions leading to different microbial communities consequently alter decomposition itself. Long-term research should be considered to simultaneously follow microbial communities and decomposition in logs over time.

ACKNOWLEDGEMENTS

This study was supported by the National Natural Science Foundation of China (31570445, 31570601, 31270498). Special thanks to Ms. Mengyun Kang for her advice on drawing the figure illustrating the experimental design and sampling.

Compliance with Ethical Standards

Conflicts of interest The co-authors of the manuscript have no conflicts of interest related to this study.

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